REMARKS

The specification has been amended to refer to the respective nucleotide- and amino acid sequences in the sequence listing by SEQ ID Numbers. These amendments have been made in order to conform with the provisions of 37 C.F.R. § 1.821(d). A marked up version of the paragraphs in the specification which have been amended herein, with additions indicated by underlining, is attached hereto in Exhibit A. No new matter has been introduced by the above-made amendments.

Applicants respectfully request entry of the amendments and remarks into the file for the above-identified application.

Respectfully submitted,

by: Jacqueline Benn
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Date April 12, 2002

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Enclosures

EXHIBIT A
A MARKED VERSION OF PARAGRAPHS IN THE SPECIFICATION AMENDED IN THE AMENDMENT FILED ON APRIL 2, 2002 IN U.S. APPLICATION SERIAL NO. 09/724,379 (ATTORNEY DOCKET NO. 7682-055-999)

On page 8, please replace the paragraph beginning "FIG. 1." with the following paragraph:

FIG. 1. Schematic representation of the RSV/CAT construct (pRSVA2CAT) used in rescue experiments. The approximate 100 nt long leader and 200 nt long trailer regions of RSV were constructed by the controlled annealing of synthetic oligonucleotides containing partial overlapping complementarity. The overlapping leader oligonucleotides are indicated by the 1L - 5L (SEQ ID No. 1-5) shown in the construct. The overlapping trailer nucleotides are indicated by the 1T - 9T (SEQ ID No. 6-13 and 49) shown in the construct. The nucleotide sequences of the leader and trailer DNAs were ligated into purified CAT gene DNA at the indicate XbaI and PstI sites respectively. This entire construct was then ligated into KpnI/HindIII digested pUC19. The inclusion of a T7 promoter sequence and a HgaI site flanking the trailer and leader sequences, respectively, allowed in vitro synthesis of RSV/CAT RNA transcripts containing the precise genomic sequence 3' and 5' ends.

On page 9, please replace the paragraph beginning "FIG. 3" with the following paragraph:

FIG. 3. Schematic representation of the RSV strain A2 genome showing the relative positions of the primer pairs used for the synthesis of cDNAs comprising the entire genome. The endonuclease sites used to splice these clones together are indicated; these sites were present in the native RSV sequence and were included in the primers used for cDNA synthesis. Approximately 100 ng of viral genomic RNA was used in RT/PCR reactions for the separate synthesis of each of the seven cDNAs. The primers for the first and second strand cDNA synthesis from the genomic RNA template are also shown. For each cDNA, the primers for the first strand synthesis are nos. 1-7 (SEQ ID No. 15, 16, 50, 18, 19, 21 and 22, respectively) and the primers for the second strand synthesis are nos. 1'-7' (SEQ ID No. 51, 24, 25, 26, 27, 48 and 28, respectively).

On page 11, please replace the paragraph beginning "FIG. 10" with the following paragraph:

FIG. 10. RSV L protein (SEQ ID No. 29) charged residue clusters targeted for site-directed mutagenesis. Contiguous charged amino acid residues in clusters were converted to alanines by site-directed mutagenesis of the RSV L gene using the QuikChange site-directed mutagenesis kit (Stratagene).

On pages 11 through 12, please replace the paragraph beginning "FIG. 11" with the following paragraph:

FIG. 11. RSV L protein (SEQ ID No. 30) cysteine residues targeted for site-directed mutagenesis. Cysteine residues were converted to alanine-residues by site-directed mutagenesis of the RSV L gene using the QuikChange site-directed mutagenesis kit (Stratagene).

On pages 33 through 34, please replace the paragraph beginning "The cDNAs of the 44 nucleotide leader" with the following paragraph:

The cDNAs of the 44 nucleotide leader and 155 nucleotide trailer components of RSV strain A2 (see Mink et al., Virology 185:615-624 (1991); Collins et al., Proc. Natl. Acad. Sci. 88:9663-9667 (1991)), the trailer component also including the promoter consensus sequence of bacteriophage T7 polymerase, were separately assembled by controlled annealing of oligonucleotides with partial overlapping complementarity (see Fig. 1). The oligonucleotides used in the annealing were synthesized on an Applied Biosystems DNA synthesizer (Foster City, CA). The separate oligonucleotides and their relative positions in the leader and trailer sequences are indicated in Fig. 1. The oligonucleotides used to construct the leader were:

1. 5' CGA CGC ATA TTA CGC GAA AAA ATG CGT ACA ACA AAC TTG CAT AAA C
2. 5' CAA AAA AAT GGG GCA AAT AAG AAT TTG ATA AGT ACC ACT TAA ATT TAA CT
3. 5' CTA GAG TTA AAT TTA AGT GGT ACT

4. 5' TAT CAA ATT CTT ATT TGC CCC ATT TTT TTG GTT TAT GCA AGT TTG TTG TA
5. 5' CGC ATT TTT TCG CGT AAT ATG CGT CGG TAC

(SEQ ID No. 1-5 respectively)

The oligonucleotides used to construct the trailer were:

1. 5' GTA TTC AAT TAT AGT TAT TAA AAA TTA AAA ATC ATA TAA TTT TTT AAA TA
2. 5' ACT TTT AGT GAA CTA ATC CTA AAG TTA TCA TTT TAA TCT TGG AGG AAT AA
3. 5' ATT TAA ACC CTA ATC TAA TTG GTT TAT ATG TGT ATT AAC TAA ATT ACG AG
4. 5' ATA TTA GTT TTT GAC ACT TTT TTT CTC GTT ATA GTG AGT CGT ATT A
5. 5' AGC TTA ATA CGA CTC ACT ATA ACG A
6. 5' GAA AAA AAG TGT CAA AAA CTA ATA TCT CGT AAT TTA GTT AAT ACA CAT AT
7. 5' AAA CCA ATT AGA TTA GGG TTT AAA TTT ATT CCT CCA AGA TTA AAA TGA TA
8. 5' ACT TTA GGA TTA GTT CAC TAA AAG TTA TTT AAA AAA TTA TAT GAT TTT TA
9. 5' ATT TTT AAT AAC TAT AAT TGA ATA CTG CA

(SEQ ID No. 6-14, respectively)

On pages 37 through 38, please replace the paragraph beginning "The following oligonucleotides" with the following paragraph:

The following oligonucleotides were used to construct the ribozyme/T7 terminator sequence:

5' GGT*GGCCGGCATGGTCCCAGC

3' CCA CCGGCCGTACCAGGGTCG (SEQ ID No. 31)

CTCGCTGGCGCCGGCTGGGCAACA

GAGCGACCGCGGCCGACCCGTGTG (SEQ ID No. 32)

TTCCGAGGGGACCGTCCCCTCGGT
AAGGCTCCCCTGGCAGGGGAGCCA (SEQ ID No. 33)

AATGGCGAATGGGACGTCGACAGC
TTACCGCTTACCCTGCAGCTGTCG (SEQ ID No. 34)

TAACAAAGCCCGAAGGAAGCT
ATTGTTTCGGGCTTCCTTCGA (SEQ ID No. 35)

GAGTTGCTGCTGCCACCGTTG
CTCAACGACGACGGAGGCAAC (SEQ ID No. 36)

AGCAATAACTAGATAACCTTGGG
TCGTTATTGATCTATTGGAACCC (SEQ ID No. 37)

CCTCTAAACGGGTCTTGAGGGTCT
GGAGATTTGCCCAGAACTCCCAGA (SEQ ID No. 38)

TTTTGCTGAAAGGAGGAACTA
AAAACGACTTTCCTCCTTGAT (SEQ ID No. 39)

TATGCGGCCGCGTCGACGGTA
ATACGCCGGCGEAGCTGCCAT (SEQ ID No. 40)

CCGGGCCCGCCTTCGAAG 3'
GGCCCGGGCGGAAGCTTC 5' (SEQ ID No. 41)

On pages 50 through 51, please replace the paragraph beginning "Recombinant RSVB-GF virus" with the following paragraph:

Recombinant RSVB-GF virus was characterized by RT/PCR using RSV subgroup B specific primers. Two independently purified recombinant RSVB-GF virus isolates were extracted with an RNA extraction kit (Tel-Test, Friendswood, TX) and RNA was precipitated by isopropanol. Virion RNAs were annealed with a primer spanning the RSV region from nt 4468 to 4492 and incubated for 1 hr under standard RT conditions (10 μl reactions) using superscript reverse transcriptase (Life Technologies, Gaithersburg, MD). Aliquots of each reaction were subjected to PCR (30 cycles at 94 C for 30 s, 55 C for 30 s and 72 C for 2 min) using subgroup B specific primers in G region (CACCACCTACCTTACTCAAGT and TTTGTTTGTGGGTTTGATGGTTGG (SEQ ID No. 42 and 43, respectively)). The PCR products were analyzed by electrophoresis on 1% agarose gel and visualized by staining with ethidium bromide. As shown in Fig. 5, no DNA product was produced in RT/PCR reactions using RSV A2 strain as template. However, a predicted product of 254 bp was detected in RT/PCR reactions utilizing RSVB-GF RNA or the PCR control plasmid, pRSVB-GF DNA, as template, indicating the rescued virus contained G and F genes derived form B9320 virus.

On page 51, please replace the paragraph beginning "RSV subgroup B strain B9320" with the following paragraph:

RSV subgroup B strain B9320 G gene was amplified from B9320 vRNA by RT/PCR and cloned into pCRII vector for sequence determination. Two Bgl II sites were incorporated into the PCR primers which also contained gene start and gene end signals (GATATCAAGATCTACAATAACATTGGGGCAAATGC and GCTAAGAGATCTTTTTGAATAACTAAGCATG (SEQ ID No. 44 and 45, respectively)). B9320G cDNA insert was digested with Bgl II and cloned into the SH/G (4630 nt) or F/M2 (7552 nt) intergenic junction of a A2 cDNA subclone (Fig. 4B and Fig. 4C). The Xho I to Msc I fragment containing B9320G insertion either at SH/G or F/M2 intergenic region was used to replace the corresponding Xho I to Msc I region of the A2 antigenomic cDNA. The resulting RSV antigenomic cDNA clone was termed as pRSVB9320G-SH/G or pRSVB9320G-F/M2.

On page 52, please replace the paragraph beginning "Expression of the inserted RSV B9320 G gene" with the following paragraph:

Expression of the inserted RSV B9320 G gene was analyzed by Northern blot using a 32P-labeled oligonucleotide specific to A2-G or B-G mRNA. Total cellular RNA was extracted from Hep-2 cells infected with wild-type RSVB 9320, rRSVA2, or rRSVB9320G-F/M2 48 hours postinfection using an RNA extraction kit (RNA stat-60, Tel-Test). RNA was electrophoresed on a 1.2% agarose gel containing formaldehyde and transferred to a nylon membrane (Amersham). An oligonucleotide specific to the G gene of the A2 stain (5'TCTTGACTGTTGTGGATTGCAGGGTTGACTTGACTCCGATCGATCC-3', SEQ ID No. 46) and an oligonucleotide specific to the B9320 G gene (5'CTTGTGTTGTTGTTGTATGGTGTGTTTCTGATTTTGTATTGATCGATCC-3', SEQ ID No. 47) were labeled with 32P-ATP by a kinasing reaction known to those of ordinary skill in the art. Hybridization of the membrane with one of the 32P-labeled G gene specific oligonucletodies was performed at 65 C and washed according to standard procedure. Both A2-G and B9320-G specific RNA were detected in the rRSVB9320G-F1M2 infected Hep-2 Cells. (Figure 6B) These results demonstrate subtype specific RNA expression.